Synaptic AMPA receptor composition in development, plasticity and disease

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Preface

AMPARs are assemblies of four core subunits, GluA1-4, that mediate most fast excitatory neurotransmission. The component subunits determine the functional properties of AMPARs and the prevailing view is that the subunit composition also determines AMPAR trafficking, which is dynamically regulated during development, synaptic plasticity, and in response to neuronal stress in disease. Recently, the subunit-dependence of AMPAR trafficking has been questioned leading to a reappraisal of the field. Here we review what is known, uncertain, conjectured and unknown about the roles of individual subunits and how they impact on AMPAR assembly, trafficking and function under normal and pathological conditions.

Introduction

AMPA receptors (AMPARs) are a subtype of ionotropic glutamate receptors that are the 'work-horses' of fast excitatory neurotransmission in the CNS. Developmentally- and activity-regulated changes in the numbers and properties of AMPARs localized at the postsynaptic membrane are essential for excitatory synapse formation, stabilization, synaptic plasticity and neural circuit formation. Consequently, the logistics of the delivery, retention and removal of individual AMPARs with defined subunit compositions at specific synapses is highly complex. A typical cortical or hippocampal pyramidal neuron contains on the order of 10,000 synapses and the AMPARs at each synapse are independently and dynamically regulated in response to developmental cues, synaptic activity and environmental stresses. Furthermore, defects in the processes that control AMPAR assembly, trafficking and synaptic expression are intimately linked to psychiatric and neurological conditions, and also with cognitive decline in neurodegenerative diseases.

Remarkable progress has been achieved in understanding how AMPAR trafficking, is orchestrated by a large array of AMPAR interacting proteins. These studies have established a set of hierarchical subunit-specific rules that control AMPAR trafficking under basal and activity-dependent conditions. Furthermore, there has been a growing appreciation that subunit composition can tune the properties of AMPARs to specific conditions. Nonetheless, how AMPARs comprising different subunits are differentially trafficked to control synaptic development, stabilisation and plasticity is a key unanswered question. Here, we provide an overview of the current state of knowledge of subunit-specific AMPAR trafficking and outline what we believe to be the key unresolved questions in the field.

Subunit-specific trafficking

Most AMPARs are heterotetrameric assemblies of combinations of the subunits GluA1, GluA2, GluA3 and GluA4. AMPARs are expressed in both neurons and glia throughout the CNS [1] and have a turnover time of between 10 hours and 2 days depending on the type of neuron and developmental stage [2, 3]. Each subunit has an identical membrane topology and core structure comprising ~900 amino acids with a molecular weight of ~105 kDa. The N-terminus is extracellular, there are three membrane spanning and one re-entrant loop domains and finally the intracellular C-terminal domain. This C-terminal domain is a highly variable region and provides a platform for both the protein interactions and posttranslational modifications that regulate subunit dependent trafficking and regulation (for reviews see [4-6]; **Fig. 1**).

Immuno-gold staining and electron microscopy studies have estimated that GluA3 is present at only ~10% of GluA1 or GluA2 levels [7]. Moreover, single cell deletion studies reported that ~80% of synaptic AMPARs in CA1 hippocampal neurons comprise GluA1/GluA2 hetromers [8]. However, other studies of subunit abundance in rat hippocampus and cortex suggest AMPARs comprise mainly heteromers containing GluA1 and GluA2 or GluA2 and GluA3 [8-10] with approximately equivalent amounts of each heteromer complex [11]. GluA4, on the other hand is tightly developmentally regulated and is sparsely expressed at glutamatergic synapses in principal neurons in adult brain [12]. It should be noted, however, that GluA4 is a key determinant of the properties of AMPAR-mediated transmission in interneurons, especially in parvalbumin-containing inhibitory interneurons [13, 14].

Research over the last 20 years has resulted in the prevailing view that a canonical hierarchy of subunit-specific rules coordinates the properties and trafficking of AMPARs, in a manner dependent on their intracellular C-termini. GluA1 is dominant in activity-dependent recruitment of AMPARs to synapses. This is mediated by CaMKII phosphorylation of GluA1 and interaction with synaptic PDZ domain-containing proteins that recruit and retain GluA1/GluA2 AMPARs at synapses during induction of long-term potentiation (LTP) [15, 16]. Once the GluA1-mediated increase in AMPAR number is established, interactions with GluA2 take over to control constitutive and activity-dependent AMPAR endocytosis, and long-term depression (LTD) [10, 17]. This model fits well with the general concept that the differential trafficking of individual subunits plays fundamentally important roles in the regulation of excitatory synapses. However, as discussed below, this model has been challenged by several recent reports. These include reports interrogating the roles in activity-dependent trafficking and synaptic plasticity of specific AMPAR subunits [18, 19], their C-terminal tails and PDZ ligands [20, 21] and changes to their phosphorylation status [22]. Taken together these findings have initiated a reappraisal of the core mechanisms underlying synaptic incorporation of AMPARs.

Subunit independent trafficking

The concept that subunit composition is the defining factor in the AMPAR trafficking underlying plasticity has been called into question by acute AMPAR subunit knockdown and molecular replacement studies [18, 19]. Contrary to expectation, using a strong LTP induction protocol it was reported that no specific AMPAR subunit is required for LTP. Remarkably, LTP still occurred even if all AMPAR subunits were removed and replaced with kainate receptors (KARs). These results suggest that the only fundamental requirement for LTP is the availability of sufficient numbers of extrasynaptic surface expressed receptors (of any type) that can be recruited to the postsynaptic membrane to increase responsiveness to glutamate released from the presynaptic terminal. At face value, these data directly contradict the prevailing dogma that recruitment of GluA1 is absolutely necessary for initial stages of LTP and

that specific point mutations within the C-terminus of GluA1 prevent plasticity [23, 24].

Clearly, further work is required to reconcile this controversy. One possible explanation is that under normal circumstances subunit-specific AMPAR trafficking pathways predominate, but that very intense saturating LTP protocols can drive subunit-independent plasticity. Nonetheless, if future studies confirm that any AMPAR, or even KAR, subunit can substitute for any other AMPAR subunit then, although there may be a role for subunit-specific mechanisms in AMPAR trafficking, at the most basic level the core processes of LTP and LTD must be subunit independent. Moreover, this would mean that synaptic plasticity is not reliant on the interacting proteins that differentially recognize different AMPAR subunits. If this is indeed the case, conceptually the most straightforward process for regulating the numbers of synaptic AMPARs is by changing the underlying synaptic organization to increase or decrease the numbers of synaptic AMPARs that can be accommodated at the postsynaptic membrane. In this scenario it would be the manipulation of the number of place-holders (or slot proteins), not the AMPARs themselves, that dictates plasticity (**Fig. 2**).

In this model the essential underlying mechanisms of functional plasticity would require ultrastructural rearrangement to recruit and retain, or release and remove ligand-gated glutamate receptors at the postsynaptic density, bypassing the need for an AMPAR subunit-specific process. An obvious example of candidate place-holders are the membrane-associated guanylate kinase (MAGUK) proteins, a family of large PDZ-domain containing scaffolds, of which PSD95 is the prototypical member. Indeed, photoactivated localization microscopy (PALM) and single-molecule immunolabeling have shown that AMPARs colocalise with dense and dynamically regulated nanodomains of PSD95 at the postsynapse in living neurons [25, 26]. However, since there are about 5-fold more PSD95 scaffolds than AMPARs at synapses [27] and CaMKII, which is essential for LTP, actually destabilizes synaptic PSD95 [28] it seems implausible that simply increasing the amount of synaptic PSD95 underlies LTP. Thus, the place-holders are most likely not individual proteins but a combination of proteins that allow the activity-dependent recruitment and stabilization of synaptic AMPA (or other) receptors.

An emerging model for synaptic retention of AMPARs involves the transmembrane AMPA receptor regulatory proteins (TARPs), AMPAR auxiliary subunits that regulate important aspects of receptor trafficking, channel activity and pharmacology [29]. Due to incompatible PDZ ligands, AMPARs do bind directly to the synaptic scaffold protein PSD95. Instead, TARPs bind to PSD95-like MAGUKs to stabilize the AMPAR/TARP complex at synapses [30]. The prototypical TARP is gamma-2 (TARP γ-2, or Stargazin), which was initially identified in the naturally occurring mutant stargazer mouse that lacks functional AMPARs at cerebellar granule cell synapses [31, 32]. Upon LTP induction, CamKII-mediated phosphorylation of Stargazin increases its binding to PSD95, providing a model for how LTP induction may be coupled to retention of synaptic AMPARs, and supporting the view that LTP is mediated by an increased affinity of AMPARs for the underlying synaptic architecture [33, 34]. However, given that TARPs do not bind KARs and that, unlike the situation for AMPARs, activation of CaMKII increases KAR lateral mobility [35], how this model can explain the ability of KARs to substitute for AMPARs in their absence is unclear.

In addition to the TARPs, the discovery that multiple synaptic transmembrane adhesion molecules, such as N-cadherin [36, 37] and the LRRTM family [38, 39] are able to cluster AMPARs raises the possibility that these proteins are also candidate place-holders, and suggests that different modes of receptor recruitment may prevail depending on the synapse and available population of extrasynaptic receptors.

Importantly, the subunit-dependent and subunit-independent modes of AMPAR trafficking are not necessarily mutually exclusive. A widely accepted model suggests

that during LTP laterally diffusing receptors are captured at synapses, and extrasynaptic receptors replenished by exocytosis of new receptors (**Fig. 2**) [40]. Thus, while synaptic capture may be flexible enough to incorporate all types of glutamate receptors, under normal conditions where AMPARs are present, subunit and TARP-dependent trafficking rules likely control LTP induction and stabilisation. That said, given the radical nature of this subunit-independent AMPAR trafficking concept, and the fact that the identities of the proteins involved is not known, these issues will undoubtedly be the focus of concerted future research, and we anticipate that the main points of controversy will be resolved in the relatively near future.

Ca^{2+}-permeable AMPARs

In adult brain the overwhelming majority of AMPARs contain an RNA-edited form of the GluA2 subunit that renders the ion channel Ca^{2+}-impermeable (CI-AMPARs; **Box 1**). Although relatively uncommon, Ca^{2+}-permeable AMPARS (CP-AMPARs) are also present in mature neurons [9] and, as discussed below, their expression can be dynamically regulated under basal, activated and stressed conditions.

While CP-AMPARs can arise from either the lack of GluA2 or the presence of an unedited GluA2 in the receptor complex, the fact that less than 1% of all GluA2 RNA encodes unedited GluA2(Q) in adult brain [41] argues that most CP-AMPARs lack GluA2. Nonetheless, some reports propose that CP-AMPARs containing unedited GluA2 can contribute to excitotoxicity (for review see [42]).

CP-AMPARs in synaptic plasticity

Many reports have implicated the regulated expression of CP-AMPARs in electrophysiologically evoked synaptic plasticity (reviewed in [43-46]) and it is becoming increasingly clear that plasticity of CP-AMPARs also occurs during various behavioural paradigms *in vivo* (**Box 2**). An emerging consensus is that the transient expression of CP-AMPARs contributes to the induction, but not the maintenance, of LTP. More specifically, several studies have suggested that LTP stimulation in 3 week-old rat hippocampal CA1 neurons evokes an initial insertion of homomeric GluA1 into the postsynaptic membrane. Experiments using philanthotoxin-433 (PhTx), which selectively blocks GluA2-lacking AMPARs [47], showed that PhTx application during and immediately after LTP induction prevents LTP expression. However, after LTP has been established PhTx had no effect, indicating that LTP requires the transient incorporation of GluA2-lacking CP-AMPARs, which are then replaced with GluA2-containing CI-AMPARs [48-50].

During LTP induction CP-AMPARs are recruited to synapses from perisynaptic pools to contribute to enhanced synaptic Ca^{2+} entry [49-51]. The Ca^{2+} gated through CP-AMPARs then plays a role in recruiting CI-AMPARs. CP-AMPAR gated Ca^{2+} also facilitates structural plasticity by activation of the small GTPase Rac1 and the downstream PAK-LIM kinase pathway, which increases spine size via modulation of actin dynamics [52]. In this model, until CP-AMPARs are replaced by CI-AMPARs, LTP remains labile and easily reversed. It should be noted, however, that the involvement of CP-AMPARs in LTP is not universally accepted [5, 53-55]. Thus, whether, how, and to what effect CP-AMPARs are incorporated during LTP remains an active area of research.

In addition to LTP, several studies have also reported specific trafficking of CP-AMPARs during LTD. For example, selective removal of CP-AMPARs mediates a specific form of depolarization-induced LTD, which only occurs in neonatal synapses [56]. Furthermore, while the presence of CP-AMPARs in hippocampal neurons under basal conditions remains controversial, it has been reported that the removal of extrasynaptic CP-AMPARs in CA1 pyramidal neurons is associated with LTD [51].

A change in the calcium permeability of AMPARs mediates a form of synaptic plasticity in granule cell-stellate cell synapses in the cerebellum. Unusually, postsynaptic AMPARs at this synapse are predominantly calcium-permeable [57]. However, high-frequency stimulation causes a change in rectification, EPSC amplitude and sensitivity to PhTx, indicative of a switch to GluA2-containing CI-AMPARs [57]. Since extrasynaptic AMPARs in stellate cells are GluA2-containing, it has been proposed that this form of plasticity occurs via Ca^{2+} influx through CP-AMPARs [57] and mGluR-dependent [58] recruitment of GluA2-containing CI-AMPARs from extrasynaptic sites (see below).

Suppression of synaptic activity increases synaptic AMPARs by homeostatic synaptic scaling [59]. The initial phases of scaling are mediated by CP-AMPARs since more GluA1 than GluA2 is recruited to synapses [60, 61]. Furthermore, CP-AMPAR selective inhibitors block the increase in synaptic current and suppression of synaptic activity is associated with local dendritic translation of GluA1, providing a rapid source of AMPARs, and suggesting the inserted CP-AMPARs are likely GluA1 homomers [62].

Other mechanisms for inducing synaptic scaling including application of tumor necrosis factor-alpha (TNFα) [63] or ablation of Arc/arg3.1 [64] also selectively recruit GluA2-lacking CP-AMPARs to synapses, but the mechanisms underlying this subunit-specific trafficking have not yet been defined. It should be noted, however, that despite these reports, several studies have failed to detect the specific incorporation of CP-AMPARs during homeostatic scaling, e.g. [65], suggesting this specific incorporation of CP-AMPARs may be dependent on the synapse, developmental stage and mode of induction. Thus, as for the proposed involvement of CP-AMPARs in LTP, the differential recruitment of CP-AMPAR during homeostatic scaling remains controversial.

Subunit expression during development

The profiles of AMPAR subunit expression and receptor assembly change markedly during development. Most notably, early in development many synapses contain GluA2-lacking, Ca^{2+}-permeable AMPARs (CP-AMPARs), which are exchanged for GluA2-containing CI-AMPARs after the second postnatal week [66]. GluA2 expression is low compared to GluA1 soon after birth, consistent with GluA2-lacking CP-AMPARs being important for neonatal synaptic function [67]. Predominant expression of GluA1 is highly developmentally restricted and in rat almost all AMPAR positive synapses express GluA2 14 days after birth (P14) [68]. Interestingly, specific factors secreted from astrocytes directly affect the subunit composition and surface expression of synaptic AMPARs during development (for review see [69]).

It has also been reported that, early in development, GluA4 homomers are preferentially inserted into silent synapses at P5–7 in an activity- and NMDAR-dependent, but CaMKII–independent manner [12]. These GluA4 homomers are subsequently exchanged for GluA2-containing receptors by a constitutive process that maintains synaptic strength. Thus, GluA4 trafficking underpins GluA1-independent developmental LTP and provides the mechanism for delivering AMPARs to previously silent synapses [12]. Furthermore, there is another shift in the composition of AMPARs by P21, as GluA3 levels increase and GluA1 levels decline. Since AMPARs containing GluA3 show reduced deactivation and desensitization compared to GluA1-containing AMPARs [70], this likely accounts for the developmental increase in the duration of AMPAR responses, postsynaptic excitability and the reduction in LTP threshold [71].

RNA editing, AMPAR assembly and ER exit

AMPAR tetramers are formed in the endoplasmic reticulum (ER) as a dimer of dimers [72, 73]. The initial dimerization of two subunits is dependent on interactions between their N-terminal domains (NTD) followed by a second dimerization step mediated by associations at the ligand binding and membrane domains [74]. The default mode is initial heterodimeric assembly, which preferentially incorporates GluA2 into the nascent receptor. However, there is considerable flexibility in the NTD dimer interface that allows the formation of GluA2-lacking AMPARs when appropriate to the relevant physiological and pathological conditions. [75].

Most assembled AMPAR tetramers contain GluA2 [9] and this is strongly regulated by Q/R editing of the GluA2 subunit. GluA1 is not edited and in the absence of GluA2 assembles into CP-AMPARs that can be rapidly exported from the ER and trafficked to the plasma membrane [76]. Unedited GluA2(Q607), where it exists, also traffics rapidly through the ER to the plasma membrane. By contrast, edited GluA2(R607), which accounts for more than 99% of GluA2, is largely unassembled and retained within the ER. Thus, in studies primarily using exogenous expression of GluA1 or GluA2, Q/R editing reduces the formation of GluA2 homotetramers and only allows GluA2 ER exit when it is assembled with GluA1. AMPARs containing both GluA1 and GluA2 follow GluA1 trafficking rules and override the ER retention of GluA2. Thus, GluA1/2 heteromers rapidly traffic from the ER to the surface whereas AMPARs without GluA1 or GluA4 transit much more slowly [77, 78] (**Fig. 3**). It is important to stress, however, that the possible roles of GluA3 in ER exit have not been systematically investigated.

Therefore, in addition to making GluA2-containing AMPARs Ca^{2+}-impermeable, Q/R editing promotes interaction with GluA1 to form heterotetrameric channels [79]. This regulated ER exit limits the types and numbers of AMPARs available for synapses and, by disfavouring GluA2 homotetramer formation, maintains a stable ER pool of edited GluA2, which is required for the formation of GluA2-containing heteromeric AMPARs later in development [80, 81].

Importantly, because it takes longer for edited GluA2 to incorporate into assembling AMPARs, the reduced ER dwell time of unedited GluA2 facilitates the rapid forward traffic and surface expression of CP-AMPARs during synapse formation and stablisation early in development [82]. As the CNS matures, developmentally controlled RNA editing of GluA2 progressively hinders its homodimerisation and retards ER exit, which then increases the incorporation of GluA2 into AMPAR heteromers, consistent with the switch from CP-AMPARs to CI-AMPARs during brain development. Interestingly, it has recently been shown that GluA2 forward trafficking is also regulated intracellular Ca^{2+} release, CaMKII activation and interaction with PICK1 to facilitate ER exit, which may imply synaptic control of forward trafficking in neurons [83].

The TARP and cornichon families of AMPAR auxiliary subunits (see below) also influence AMPAR assembly and forward traffic. More specifically, TARPs act as chaperones that prevent ER exit of incorrectly folded AMPARs [84]. Moreover, TARPs are essential for ER export of correctly assembled receptors [85] and remain part of the AMPAR complex throughout Golgi processing and forward trafficking to eventual surface expression at the postsynaptic density [86]. Similarly, cornichons have a well-defined role in the export of specific proteins from the ER [87, 88] including AMPARs [89, 90].

Mechanisms of subunit-specific trafficking

Role of interacting proteins

Individual AMPAR subunits have either long or short intracellular C-terminal domains (tails) that bind to distinct sets of interacting proteins. Remarkable progress has been achieved in identifying how these interacting proteins impact on AMPAR biosynthesis, trafficking, scaffolding, stability, signaling, and turnover (for reviews [4-6]). The main

splice isoforms of GluA1 and GluA4 have long-tails and GluA2 and GluA3 have short-tails. Generally the long-tailed GluA1 and GluA4 subunits dictate the trafficking properties of AMPARs when assembled in heteromers with short-tailed subunits and, since GluA4 is expressed mainly during early development, GluA1 is by far the predominant long-tailed subunit in mature neurons. The observation that CP- and CI-AMPARs are differentially trafficked during several forms of plasticity raises the question of how these receptors are distinguished by interacting proteins to promote or reduce synaptic incorporation of particular subtypes. Many aspects remain to be resolved but core concepts of how this is achieved are beginning to emerge (**Fig. 4**).

The PDZ domain-containing protein PICK1 interacts with GluA2 and GluA3 subunits through their C-terminal PDZ ligands [91]. Early studies showed that PICK1 overexpression increases synaptic CP-AMPARs and blocks LTP at CA3-CA1 synapses [92]. In contrast, a subsequent study reported that PICK1 played no role in hippocampal LTP, instead suggesting that it is involved in AMPAR recycling [93] and LTD through promoting the intracellular retention of internalized AMPARs [94]. However, these discrepancies may be explained, at least in part, by the requirement of PICK1 in LTP being dependent on the developmental stage and the induction protocol used [95]. Furthermore, since LTP relies on recycling of synaptic AMPARs [96] the effects of PICK1 on LTP may be via regulation of GluA2 recycling. Indeed, using glycine-induced LTP in neuronal cultures as a model system it has been proposed that PICK1 can specifically restrict the recycling of GluA2-containing CI-AMPARs upon LTP induction, thereby promoting the switch to synaptic GluA1-homomeric CP-AMPARs [50]. Since GluA1 insertion is required for LTP [16] this mechanism of restricting GluA2 recycling is likely additive to interactions with GluA1 that promote synaptic incorporation of this subunit during LTP.

The direct role of GluA1 interactors in promoting synaptic incorporation of CP-AMPARs is largely unclear. Indeed, while early studies reported the requirement for the GluA1 PDZ ligand in mediating insertion of GluA1-containing AMPARs during LTP [16], this observation has been called into question by the generation of knock-in mice lacking the final 7 C-terminal residues of GluA1, including the PDZ ligand [20]. At CA1 hippocampal synapses, neither localization of GluA1 nor LTP or LTD were altered by deletion of the GluA1 PDZ ligand. Furthermore, high-resolution imaging has determined that GluA1 synaptic mobility and distribution does not require its PDZ ligand [21, 33]. Thus it seems that direct PDZ interactions with the GluA1 cytoplasmic tail are not required for synaptic plasticity. Rather, the predominant interaction mediating GluA1 synaptic recruitment and retention during LTP is mediated through TARP binding to PSD95 [30, 33, 97]. More specifically, CaMKII phosphorylation of Stargazin enhances its binding to PSD95, to promote synaptic retention of GluA1-containing AMPARs [33, 98], consistent with preferential insertion of GluA1 during LTP. However, given that Stargazin binds all AMPAR subunits [31], how this occurs is unclear. Thus, although direct PDZ interactions with the C-terminal ligand of GluA1 are not absolutely required for LTP, they likely play a key modulatory role during synaptic delivery of GluA1-containing AMPARs during LTP [99]. For example, the GluA1 PDZ ligand binds selectively to Synapse-associated protein 97 (SAP97), which delivers GluA1-containing AMPARs to synapses following CaMKII activation via binding to the motor protein myosin VI [100, 101]. Furthermore, the endosomal PDZ domain-containing protein SNX27 binds both GluA1 and GluA2 and is involved in both maintaining basal AMPAR surface expression in addition to mediating AMPAR insertion during LTP [102, 103].

Plasticity at the cerebellar granule cell-stellate cell synapse is characterized by the replacement of synaptic CP-AMPARs with CI-AMPARs (**Fig. 5**). Despite this process being the reverse of that observed during hippocampal LTP, PICK1 is also required for this form of plasticity [104, 105]. In contrast to its proposed role in restricting surface expression of GluA2-containing AMPARs during hippocampal LTP, PICK1 promotes the CP- to CI-AMPAR switch in stellate cells by supporting an extrasynaptic pool of

GluA2-containing receptors, although the molecular mechanisms underlying this effect are unclear. Furthermore, another PDZ domain-containing GluA2/3 interacting protein, GRIP1, has also been implicated in this process. GRIP1 is thought to anchor AMPARs at synapses [106]. Peptides that block the GRIP1-GluA2/3 interaction destabilize GluA2-lacking CP-AMPARs at synapses, and synaptic activity reduces the interaction of GRIP1 with CP-AMPARs [104]. Given that synaptic CP-AMPARs in stellate cells have been suggested to be GluA3 homomers [45], this implies specific regulation of the interaction of GRIP1 with GluA2 versus GluA3 and further work will be required to define how this is achieved.

The multimeric ATPase NSF has also been implicated in stellate cell plasticity [105]. NSF interacts with GluA2 and blockade of the GluA2-NSF interaction results in a rapid run-down in AMPAR EPSCs, supporting a role for NSF in constitutive cycling of GluA2-containing AMPARs [107]. In stellate cells, blocking the GluA2-NSF interaction did not affect the extrasynaptic pool of GluA2, but did prevent the activity-dependent switch between CP- and CI-AMPARs. These data suggest that NSF is specifically required for the synaptic incorporation of GluA2-containing receptors but, again, the molecular mechanisms underlying this remain to be determined.

AMPAR auxiliary subunits

The discovery of multiple auxiliary subunits with overlapping roles has greatly extended our understanding of the diversity of AMPAR macromolecular complexes (reviewed in [32, 108, 109]. Currently identified AMPAR auxiliary subunits include TARPs, suppressor of lurcher (SOL) [110], Cornichon homologues (CNIHs) [111], synapse differentiation-induced genes (SynDIG I and SynDIG4) [112, 113], cysteine-knot AMPAR modulating protein family [114, 115], and germ cell-specific gene 1-like (GSG1L) protein [116]. Of these the prototypical TARP family of AMPAR auxiliary subunits are by far the best characterized.

There are seven TARPs, γ2 to γ8 (for review see [108]). A large body of work has demonstrated that Stargazin, and other Type I TARPs (γ3, γ4 and γ8), can promote synaptic trapping of AMPARs through binding to PSD95 and, as discussed above, this interaction offers an attractive mechanism for how AMPARs are recruited and accumulated at the postsynapse during LTP [33, 98]. Furthermore, dynamic AMPAR-TARP interactions have also been recently demonstrated to underlie by ability of AMAPRs to support high-frequency stimulation despite undergoing desentisiation [117]. A general consensus is that agonist binding reduces AMPAR affinity for Stargazin [98, 117, 118] (but see [119, 120]) allowing AMPARs to diffuse away from the synaptically-anchored Stargazin. This loss of desensitised AMPARs from the postsynaptic density frees up ‘slots’ for non-desensitised AMPARs, which maintains synaptic transmission [117]. Thus, in addition to their well-characterised role in determining the biophysical properties of AMPARs, TARPs also play a key role in synaptic AMPAR retention under basal and activity-dependent conditions.

Beyond the synaptic trapping of AMPARs, the TARPs Stargazin and γ5 directly reduce the polyamine sensitivity of CP-AMPARs [121, 122]. Because polyamine block is a parameter used to determine the GluA2 content of the receptors, the presence or absence of TARPs can therefore complicate unambiguous determination of subunit content and necessitates careful consideration of the role of TARPs in the trafficking of CP- versus CI-AMPARs. Nonetheless, in Stargazer mice there is a marked increase in the CP-AMPAR component of synaptic responses in cerebellar stellate cells, indicating that, unlike CI-AMPARs, CP-AMPARs are synaptically expressed in the absence of γ2. Consistent with the absence of γ2 these synaptic CP-AMPARs exhibited low conductance and were blocked by spermine [123] (but see [124]). In cerebellar granule cells, another TARP, γ7, promotes surface expression of CP-AMPARs while restricting surface expression of CI-AMPARs [125]. Interestingly, however, neither γ7-associated CP-AMPARs nor CI-AMPARs are synaptically expressed unless the complex also contains γ2. Thus, while the role of TARP association in plasticity of CP-AMPARs has

not been fully elucidated, it is clear that TARP association can promote trafficking of CP- versus CI-AMPARs and that the levels and properties of synaptic AMPARs are intricately controlled by association with TARPs.

Given the expanding repertoire of AMPAR auxiliary subunits [32, 109], and the discovery that cornichon proteins can determine the subunit composition of synaptic AMPARs by promoting incorporation of GluA1-containing receptors [126] an important question for future work will be to examine how these newly-discovered proteins orchestrate the properties and synaptic incorporation of CP- versus CI-AMPARs. Indeed, even for TARPs and cornichons, many of the specific mechanisms of action remain to be determined, and how the effects of the different auxiliary summate or occlude each other is entirely unknown. Thus, defining how, where and when AMPAR auxiliary subunits assemble with AMPARs and how they interact and/or compete is likely to be a fruitful avenue for future research.

AMPAR phosphorylation

The kinases and phosphorylation sites on different AMPAR subunits, and their roles in receptor trafficking, plasticity and function have been intensively studied and extensively reviewed (for recent reviews see [6, 127, 128]).

CaMKII, PKA and PKC are each critical modulators of LTP and LTD. In general, increased phosphorylation leads to LTP and decreased phosphorylation leads to LTD [129, 130]. CaMKII and PKC can phosphorylate the GluA1 subunit at S831 [131], which increases the conductance of homomeric GluA1 and GluA1/2 heteromers in the presence of TARPs [132], while PKA phosphorylates GluA1 at S845, increasing the peak conductance and open probability of the channel [133]. Knock-in mice expressing GluA1 or GluA2 subunits mutated to non-phosphorylatable or phosphomimetic residues have demonstrated that phosphorylation of GluA1 by CaMKII or PKA is necessary for full hippocampal LTP expression [134], while dephosphorylation of the PKA site in GluA1 is required for LTD [46]. Furthermore, PKC phosphorylation of GluA2 is required for cerebellar LTD [135]. Beyond GluA1 and GluA2, transient expression of GluA4 in CA1 pyramidal neurons during early development underpins the switch in kinase signaling in LTP from PKA to CaMKII-dependent mechanisms [136]. PKA activation drives synaptic expression of GluA4, and PKA-mediated recruitment of GluA4-containing AMPARs in immature synapses unsilences silent synapses [136].

Surprisingly, recent analysis of the stoichiometry of GluA1 phosphorylation using Phos-tag SDS-PAGE indicates only ~1% of total GluA1 is phosphorylated at S831 and less than 0.1% is phosphorylated S845. The estimated number of GluA1-containing AMPARs at a given synapse is ~100 so, on average, very few synapses will contain any GluA1 phosphorylated at either S831 or S845. No GluA1 simultaneously phosphorylated at both S831 and S845 were detected [22]. The fraction of GluA1 phosphorylated at these residues was increased by activity but it still remained extremely low, leading the authors to question whether this level of phosphorylation can support its proposed role in AMPAR trafficking.

These results are puzzling since synaptic plasticity is impaired in non-phosphorylatable S831A/S845A knock-in mutant GluA1 mice [23]. The authors propose one possible explanation is that phosphorylation of a very small proportion of GluA1 might trigger changes in adjacent unphosphorylated AMPARs by an unknown mechanism. While further work is required, it is notable that due to their dynamic nature, other posttranslational modifications such as ubiquitination and SUMOylation affect only a very small proportion of substrate at any one time while still having a major effect on the substrate pool [137].

Notwithstanding the results of the Phos-tag study [22], many reports have examined how phosphorylation differentially traffics CP- versus CI-AMPARs and accumulating evidence points to PKA-mediated phosphorylation of S845 in GluA1 as an important determinant in GluA1-containing CP-AMPAR trafficking in hippocampal LTP, LTD

and homeostatic scaling (**Fig. 4**). During hippocampal LTP, it has been proposed that CP-AMPARs are first inserted at extrasynaptic sites and then incorporated into synapses by lateral diffusion in the membrane [49]. He et al [51] observed a pool of extrasynaptic CP-AMPARs in CA1 neurons that is absent in neurons from mice where GluA1-S845 is mutated to a non-phosphorylatable alanine. This loss was attributed to lysosomal degradation of homomeric GluA1 receptors, and supports a model whereby PKA-mediated phosphorylation of GluA1 stabilises extrasynaptic GluA1 homomers, potentially priming them for synaptic incorporation during LTP. Although the authors did not examine LTP directly, they did observe that dephosphorylation of GluA1-S845 was associated with NMDAR-dependent LTD, suggesting that removal of CP-AMPARs contributes to this form of LTD, and that the phosphorylation state of GluA1-S845 may control the supply of extrasynaptic CP-AMPARs for bidirectional synaptic plasticity [51].

GluA1-S845 is dephosphorylated by the calcium-dependent phosphatase calcineurin. Calcineurin and PKA are anchored at synapses by the protein AKAP150 (the rodent orthologue of human AKAP79), and knock-in of an AKAP150 mutant defective in calcineurin binding leads to enhanced GluA1-S845 phosphorylation and increases synaptic expression of CP-AMPARs [138]. Consistent with increased GluA1-S845 phosphorylation, these mice do not exhibit LTD at CA3-CA1 synapses, but show enhanced LTP [138].

A role for GluA1-S845 phosphorylation has also been proposed in the synaptic incorporation of CP-AMPARs during synaptic scaling. Cultures from knock-in mice harbouring the non-phosphorylatable S845A mutation [139] do not undergo TTX-induced synaptic upscaling. Moreover, TTX reduces calcineurin activity and upregulates phosphorylation of GluA1-S845, and inhibition of calcineurin mimics upscaling in the absence of TTX [139]. Consistent with this, during TTX-induced scaling active PKA is enriched at synapses to mediate phosphorylation of GluA1 S845 and this process also requires the involvement of AKAP150 [140]. Together, these data highlight the critical importance of GluA1-S845 phosphorylation in controlling the availability of synaptic CP-AMPARs.

Intriguingly, although it does not directly phosphorylate AMPAR subunits, CaMKI has also been implicated in CP-AMPAR expression during hippocampal LTP [141]. Infusion of active CaMKI potentiates AMPARs in cultured neurons through synaptic incorporation of CP-AMPARs, in a manner that requires actin polymerisation. Furthermore, in slices, CA3-CA1 LTP induced by theta burst stimulation recruited CP-AMPARs, and this was prevented by inhibition of CaMKK, an upstream activator of CamKI [141]. However, as yet, the targets of CamKI that mediate synaptic expression of CP-AMPARs have not been identified.

AMPAR subunits and disease

Because of the paramount importance of correctly regulated AMPAR synaptic expression, almost any defect in the processes that control their trafficking or activity can have dire consequences on brain function. Indeed, most neurological and neurodegenerative disorders involve synaptic malfunction that can be linked to abnormalities in AMPARs [142]. For example, one of the earliest cell biological manifestations of dementia in Alzheimer's disease (AD) is reduced synaptic AMPARs, and aberrations in LTP and LTD [143]. Furthermore, disruption of AMPAR trafficking by soluble amyloid beta (Aβ) oligomers is a major causative agent of synaptic dysfunction in AD [144]. More specifically, it has been reported that Aβ oligomers bind in close proximity to GluA2-containing complexes and AMPAR antagonists inhibit Aβ oligomer binding and synaptic loss, raising the possibility that Aβ may affect AMPAR trafficking by binding directly to the GluA2 protein complex [145]. Conversely, intracellular application of oligomerised Aβ causes an acute increase in

AMPAR-mediated synaptic responses, which requires PKA phosphorylation of S845 in GluA1. Ablation of GluA1, but not GluA2, prevents this increase, consistent with intracellular Aβ causing an enhancement of synaptic CP-AMPAR number and consequent excitotoxicity [146].

The selective loss of GluA2-containing AMPARs and the subsequent increase in intracellular Ca^{2+} due to the expression of CP-AMPARs is implicated in the pathology of many other diseases. Prolonged decreases in surface GluA2-containing AMPARs in vulnerable neurons mediate a switch from CI-AMPARs to CP-AMPARs that is a causal factor in ischemic neuronal death (for review see [91]). A similar mechanism has also been proposed for synaptic dysfunction and neuronal death after traumatic brain injury [147]. Furthermore, neurons subject to epileptic seizures markedly downregulate GluA2 mRNA and subunit expression [148]. Interestingly, in addition to disease mechanisms arising from CP-AMPARs lacking GluA2, dysfunctional Q/R editing in GluA2 occurs in motor neurons of amyotrophic lateral sclerosis (ALS) patients, caused by reduced expression of the ADAR2 RNA editing enzyme, indicating a link between expression of CP-AMPARs that contain unedited GluA2, and neuronal death in ALS [149]. Together, these studies highlight the balance between CP- and CI-AMPARs as a crucial determinant of neuronal fate and, although much remains to be learned, raise the potential for modulation of this pathway as a possible strategy for therapeutic intervention in a number of disease states.

Current position and pressing questions

The dynamic regulation of AMPAR subunit composition is a critical factor in neuronal vulnerability to stress and, more controversially, may represent a core mechanism underlying physiological forms of plasticity induction. Most attention has focused on the GluA2 subunit because it has profound affects on AMPAR assembly, trafficking and the ability of the channel to gate Ca^{2+}. Moreover, in general terms, the dysregulation of GluA2 incorporation into AMPAR complexes has been strongly implicated in neuronal damage and disease.

A widely accepted model is that AMPAR trafficking and surface expression is governed by a strictly hierarchical series of rules that is dictated by interactions with the composite subunits. In this system, long-tailed GluA1 subunits dominate in heteromeric receptor complexes undergoing activity-dependent trafficking processes while, under basal conditions, AMPAR endocytosis and recycling is regulated by interactions with the short-tailed GluA2 subunit. This dogma has been recently questioned by results that appear to indicate that, although the subunit composition of AMPARs can influence trafficking and synaptic expression, it is not fundamental to these processes. These intriguing findings challenge core concepts and certainly warrant a reanalysis and reevaluation of the field. In particular, it will be important to determine under what physiological conditions activity-dependent AMPAR trafficking can occur independent of subunit composition and, where this occurs, how the incorporated receptors are recruited and stabilised at the synapse. A major focus will be the identification of place-holder proteins that can capture, retain and promote surface expression of the wide array of glutamate receptors which can support LTP under these conditions.

An emerging theory for synaptic retention of AMPARs is the binding of TARPs to PSD95, however it is unclear how this mechanism supports the subunit-specific trafficking of AMPARs observed during plasticity. Nonetheless, the realization that AMPAR auxiliary proteins also function as interaction platforms to expand the repertoire of AMPAR interacting proteins clearly warrants further investigation to identify and characterize new protein partners. These findings also demonstrate that the subunit composition and assembly of AMPARs extends far beyond just the pore-forming subunits. Analysis of the stoichiometry and competition between different

auxiliary proteins present in different receptor complexes will open new and potentially exciting avenues of investigation that will provide a molecular basis for the extraordinary flexibility of neurons to adapt synaptic transmission in response to activity.

While neurons may be able to support LTP in a subunit- and receptor subtype-independent manner under some conditions, it is clear from a wealth of studies that, under most experimental conditions, the subunit-specific rules of AMPAR trafficking prevail. However, many questions remain, even for this established model, particularly with respect to the differential trafficking of CP- versus CI-AMPARs. Although it remains controversial, mounting evidence supports a role for specific trafficking of CP-AMPARs in forms of plasticity both *in vitro* and *in vivo*. We expect that standardization of the precise experimental conditions and developmental stages used should reconcile these controversies. Nonetheless, the signalling pathways, interacting proteins and post-translational modifications that mediate the specific trafficking of CP- versus CI-AMPARs remain largely undefined. More generally, how the subunit composition of AMPARs is rapidly regulated at individual synapses also remains enigmatic. For example, are reserves of AMPARs assembled from different combinations of subunits available for insertion into the membrane under appropriate conditions and, if so, how are these complexes selected? How is the subunit composition of locally synthesized receptors controlled, and what signals promote their synaptic incorporation? Further insight into the signalling pathways and molecular determinants underlying these local events will be crucial to better understand neuronal function. Finally, the involvement of dysregulated CP-AMPAR in disease is intriguing, and offers the exciting possibility that these receptors may constitute a druggable target in a number of disorders of the nervous system. We anticipate that these questions will receive much attention in the coming years.

In conclusion, over the last three decades there have been impressive advances in our understanding of how AMPAR trafficking underpins synaptic function. Despite this progress, however, many fundamental questions remain, and the selective regulation of individual AMPAR subunits and the functional and pathological roles of CP-AMPARs are important themes for future study. Answering these questions will reveal in ever-greater detail the complex mechanistic processes that underlie brain function and provide new insights and new strategies to combat psychiatric and neurodegenerative diseases.

Author contributions

KAW and JMH contributed equally to the conception, writing and editing of this review and are co-corresponding authors.

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References

1. Wisden, W. & Seeburg, P.H. Mammalian ionotropic glutamate receptors. *Curr Opin Neurobiol* **3**, 291-8 (1993).
2. Mammen, A.L., Huganir, R.L. & O'Brien, R.J. Redistribution and stabilization of cell surface glutamate receptors during synapse formation. *J Neurosci* **17**, 7351-8 (1997).

3. Archibald, K., Perry, M.J., Molnar, E. & Henley, J.M. Surface expression and metabolic half-life of AMPA receptors in cultured rat cerebellar granule cells. *Neuropharmacology* **37**, 1345-53 (1998).
4. Henley, J.M., Barker, E.A. & Glebov, O.O. Routes, destinations and delays: recent advances in AMPA receptor trafficking. *Trends Neurosci* **34**, 258-68 (2011).
5. Huganir, R.L. & Nicoll, R.A. AMPARs and synaptic plasticity: the last 25 years. *Neuron* **80**, 704-17 (2013).
6. Nicoll, R.A. & Roche, K.W. Long-term potentiation: peeling the onion. *Neuropharmacology* **74**, 18-22 (2013).
7. Sans, N. et al. Aberrant formation of glutamate receptor complexes in hippocampal neurons of mice lacking the GluR2 AMPA receptor subunit. *J Neurosci* **23**, 9367-73 (2003).
8. Lu, W. et al. Subunit composition of synaptic AMPA receptors revealed by a single-cell genetic approach. *Neuron* **62**, 254-68 (2009).
9. Wenthold, R.J., Petralia, R.S., Blahos, J., II & Niedzielski, A.S. Evidence for multiple AMPA receptor complexes in hippocampal CA1/CA2 neurons. *J Neurosci* **16**, 1982-9. (1996).
10. Shi, S., Hayashi, Y., Esteban, J.A. & Malinow, R. Subunit-specific rules governing AMPA receptor trafficking to synapses in hippocampal pyramidal neurons. *Cell* **105**, 331-43. (2001).

This paper proposed the now widely accepted model that regulated addition of GluA1/2 and continuous replacement of GluA2/3-containing synaptic AMPARs provides the mechanism for how surface receptor number is established and maintained.

11. Kessels, H.W., Kopec, C.D., Klein, M.E. & Malinow, R. Roles of stargazin and phosphorylation in the control of AMPA receptor subcellular distribution. *Nat Neurosci* **12**, 888-96 (2009).
12. Zhu, J.J., Esteban, J.A., Hayashi, Y. & Malinow, R. Postnatal synaptic potentiation: delivery of GluR4-containing AMPA receptors by spontaneous activity. *Nat Neurosci* **3**, 1098-106 (2000).
13. Bernard, V., Somogyi, P. & Bolam, J.P. Cellular, subcellular, and subsynaptic distribution of AMPA-type glutamate receptor subunits in the neostriatum of the rat. *J Neurosci* **17**, 819-33 (1997).
14. Pelkey, K.A. et al. Pentraxins coordinate excitatory synapse maturation and circuit integration of parvalbumin interneurons. *Neuron* **85**, 1257-72 (2015).
15. Zamanillo, D. et al. Importance of AMPA receptors for hippocampal synaptic plasticity but not for spatial learning. *Science* **284**, 1805-1811. (1999).
16. Hayashi, Y. et al. Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. *Science* **287**, 2262-7 (2000).
17. Lee, S.H., Simonetta, A. & Sheng, M. Subunit rules governing the sorting of internalized AMPA receptors in hippocampal neurons. *Neuron* **43**, 221-36 (2004).
18. Granger, A.J., Shi, Y., Lu, W., Cerpas, M. & Nicoll, R.A. LTP requires a reserve pool of glutamate receptors independent of subunit type. *Nature* **493**, 495-500 (2013).

Contrary to the prevailing view, this paper reported the unexpected finding that there is no absolute requirement for any individual AMPAR subunit, or C-terminal protein interaction, for LTP.

19. Granger, A.J. & Nicoll, R.A. LTD expression is independent of glutamate receptor subtype. *Front Synaptic Neurosci* **6**, 15 (2014).
20. Kim, C.H. et al. Persistent hippocampal CA1 LTP in mice lacking the C-terminal PDZ ligand of GluR1. *Nat Neurosci* **8**, 985-7 (2005).

21. Kerr, J.M. & Blanpied, T.A. Subsynaptic AMPA receptor distribution is acutely regulated by actin-driven reorganization of the postsynaptic density. *J Neurosci* **32**, 658-73 (2012).
22. Hosokawa, T., Mitsushima, D., Kaneko, R. & Hayashi, Y. Stoichiometry and phosphoisotypes of hippocampal AMPA-type glutamate receptor phosphorylation. *Neuron* **85**, 60-7 (2015).

Using biochemical approaches this report suggests that there is almost no phosphorylation of GluA1 at S831 and S845, the two major sites identified many previous papers as critical for AMPAR regulation.

23. Lee, H.K. et al. Phosphorylation of the AMPA Receptor GluR1 Subunit Is Required for Synaptic Plasticity and Retention of Spatial Memory. *Cell* **112**, 631-43 (2003).

This study generated transgenic mice that express GluA1 with mutated phosphorylation sites that demonstrated that phosphorylation of GluR1 is required for LTD and LTP, and learning and memory.

24. Esteban, J.A. et al. PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity. *Nat Neurosci* **6**, 136-43 (2003).
25. MacGillavry, H.D., Song, Y., Raghavachari, S. & Blanpied, T.A. Nanoscale scaffolding domains within the postsynaptic density concentrate synaptic AMPA receptors. *Neuron* **78**, 615-22 (2013).
26. Nair, D. et al. Super-resolution imaging reveals that AMPA receptors inside synapses are dynamically organized in nanodomains regulated by PSD95. *J Neurosci* **33**, 13204-24 (2013).
27. Sheng, M. & Hoogenraad, C.C. The postsynaptic architecture of excitatory synapses: a more quantitative view. *Annu Rev Biochem* **76**, 823-47 (2007).
28. Steiner, P. et al. Destabilization of the postsynaptic density by PSD-95 serine 73 phosphorylation inhibits spine growth and synaptic plasticity. *Neuron* **60**, 788-802 (2008).
29. Coombs, I.D. & Cull-Candy, S.G. Transmembrane AMPA receptor regulatory proteins and AMPA receptor function in the cerebellum. *Neuroscience* **162**, 656-65 (2009).
30. Bats, C., Groc, L. & Choquet, D. The interaction between Stargazin and PSD-95 regulates AMPA receptor surface trafficking. *Neuron* **53**, 719-34 (2007).
31. Chen, L. et al. Stargazing regulates synaptic targeting of AMPA receptors by two distinct mechanisms. *Nature* **408**, 936-43. (2000).

This paper reports that AMPAR subunit interaction with stargazin is required for surface expression of AMPARs and implies the widespread influence of stargazin-like processes (TARPs) in AMPAR trafficking.

32. Sumioka, A. Auxiliary subunits provide new insights into regulation of AMPA receptor trafficking. *J Biochem* **153**, 331-7 (2013).
33. Opazo, P. et al. CaMKII triggers the diffusional trapping of surface AMPARs through phosphorylation of stargazin. *Neuron* **67**, 239-52 (2010).
34. Opazo, P., Sainlos, M. & Choquet, D. Regulation of AMPA receptor surface diffusion by PSD-95 slots. *Curr Opin Neurobiol* (2012).
35. Carta, M. et al. CaMKII-dependent phosphorylation of GluK5 mediates plasticity of kainate receptors. *EMBO J* **32**, 496-510 (2013).
36. Saglietti, L. et al. Extracellular interactions between GluR2 and N-cadherin in spine regulation. *Neuron* **54**, 461-77 (2007).
37. Tai, C.Y., Kim, S.A. & Schuman, E.M. Cadherins and synaptic plasticity. *Curr Opin Cell Biol* **20**, 567-75 (2008).
38. Linhoff, M.W. et al. An unbiased expression screen for synaptogenic proteins identifies the LRRTM protein family as synaptic organizers. *Neuron* **61**, 734-49 (2009).
39. Soler-Llavina, G.J. et al. Leucine-rich repeat transmembrane proteins are essential for maintenance of long-term potentiation. *Neuron* **79**, 439-46 (2013).

This study shows that LRRTM synaptic cell adhesion molecules are required for LTP due to maintaining the surface expression of newly recruited synaptic AMPARs.

40. Makino, H. & Malinow, R. AMPA receptor incorporation into synapses during LTP: the role of lateral movement and exocytosis. *Neuron* **64**, 381-90 (2009).
41. Kawahara, Y., Ito, K., Sun, H., Kanazawa, I. & Kwak, S. Low editing efficiency of GluR2 mRNA is associated with a low relative abundance of ADAR2 mRNA in white matter of normal human brain. *Eur J Neurosci* **18**, 23-33 (2003).
42. Wright, A. & Vissel, B. The essential role of AMPA receptor GluR2 subunit RNA editing in the normal and diseased brain. *Front Mol Neurosci* **5**, 34 (2012).
43. Isaac, J.T., Ashby, M. & McBain, C.J. The role of the GluR2 subunit in AMPA receptor function and synaptic plasticity. *Neuron* **54**, 859-71 (2007).
44. Liu, S.J. & Zukin, R.S. Ca2+-permeable AMPA receptors in synaptic plasticity and neuronal death. *Trends Neurosci* **30**, 126-34 (2007).
45. Cull-Candy, S., Kelly, L. & Farrant, M. Regulation of Ca2+-permeable AMPA receptors: synaptic plasticity and beyond. *Curr Opin Neurobiol* **16**, 288-97 (2006).
46. Man, H.Y. GluA2-lacking, calcium-permeable AMPA receptors--inducers of plasticity? *Curr Opin Neurobiol* **21**, 291-8 (2011).
47. Washburn, M.S. & Dingledine, R. Block of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors by polyamines and polyamine toxins. *J Pharmacol Exp Ther* **278**, 669-78 (1996).
48. Plant, K. et al. Transient incorporation of native GluR2-lacking AMPA receptors during hippocampal long-term potentiation. *Nat Neurosci* **9**, 602-4 (2006).
49. Yang, Y., Wang, X.B. & Zhou, Q. Perisynaptic GluR2-lacking AMPA receptors control the reversibility of synaptic and spines modifications. *Proc Natl Acad Sci U S A* **107**, 11999-2004 (2010).
50. Jaafari, N., Henley, J.M. & Hanley, J.G. PICK1 mediates transient synaptic expression of GluA2-lacking AMPA receptors during glycine-induced AMPA receptor trafficking. *J Neurosci* **32**, 11618-30 (2012).
51. He, K. et al. Stabilization of Ca2+-permeable AMPA receptors at perisynaptic sites by GluR1-S845 phosphorylation. *Proc Natl Acad Sci U S A* **106**, 20033-8 (2009).
52. Fortin, D.A. et al. Long-term potentiation-dependent spine enlargement requires synaptic Ca2+-permeable AMPA receptors recruited by CaM-kinase I. *J Neurosci* **30**, 11565-75 (2010).
53. Adesnik, H. & Nicoll, R.A. Conservation of glutamate receptor 2-containing AMPA receptors during long-term potentiation. *J Neurosci* **27**, 4598-602 (2007).
54. Gray, E.E., Fink, A.E., Sarinana, J., Vissel, B. & O'Dell, T.J. Long-term potentiation in the hippocampal CA1 region does not require insertion and activation of GluR2-lacking AMPA receptors. *J Neurophysiol* **98**, 2488-92 (2007).
55. Granger, A.J. & Nicoll, R.A. Expression mechanisms underlying long-term potentiation: a postsynaptic view, 10 years on. *Philos Trans R Soc Lond B Biol Sci* **369**, 20130136 (2014).
56. Ho, M.T. et al. Developmental expression of Ca2+-permeable AMPA receptors underlies depolarization-induced long-term depression at mossy fiber CA3 pyramid synapses. *J Neurosci* **27**, 11651-62 (2007).
57. Liu, S.Q. & Cull-Candy, S.G. Synaptic activity at calcium-permeable AMPA receptors induces a switch in receptor subtype. *Nature* **405**, 454-8 (2000).

This paper was the first description of activity-induced changes in the AMPAR subunit composition from GluA2-lacking, Ca^{2+}-permeable to GluA2-containing Ca^{2+}-impermeable complexes at cerebellar stellate cell synapses.

58. Kelly, L., Farrant, M. & Cull-Candy, S.G. Synaptic mGluR activation drives plasticity of calcium-permeable AMPA receptors. *Nat Neurosci* **12**, 593-601 (2009).
59. Turrigiano, G.G. The self-tuning neuron: synaptic scaling of excitatory synapses. *Cell* **135**, 422-35 (2008).
60. Thiagarajan, T.C., Lindskog, M. & Tsien, R.W. Adaptation to synaptic inactivity in hippocampal neurons. *Neuron* **47**, 725-37 (2005).
61. Aoto, J., Nam, C.I., Poon, M.M., Ting, P. & Chen, L. Synaptic signaling by all-trans retinoic acid in homeostatic synaptic plasticity. *Neuron* **60**, 308-20 (2008).
62. Sutton, M.A. et al. Miniature neurotransmission stabilizes synaptic function via tonic suppression of local dendritic protein synthesis. *Cell* **125**, 785-99 (2006).
63. Ogoshi, F. et al. Tumor necrosis-factor-alpha (TNF-alpha) induces rapid insertion of Ca2+-permeable alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA)/kainate (Ca-A/K) channels in a subset of hippocampal pyramidal neurons. *Exp Neurol* **193**, 384-93 (2005).
64. Shepherd, J.D. et al. Arc/Arg3.1 mediates homeostatic synaptic scaling of AMPA receptors. *Neuron* **52**, 475-84 (2006).
65. Gainey, M.A., Hurvitz-Wolff, J.R., Lambo, M.E. & Turrigiano, G.G. Synaptic scaling requires the GluR2 subunit of the AMPA receptor. *J Neurosci* **29**, 6479-89 (2009).
66. Pellegrini-Giampietro, D.E., Bennett, M.V. & Zukin, R.S. Are Ca(2+)-permeable kainate/AMPA receptors more abundant in immature brain? *Neurosci Lett* **144**, 65-9 (1992).
67. Pickard, L., Noel, J., Henley, J.M., Collingridge, G.L. & Molnar, E. Developmental changes in synaptic AMPA and NMDA receptor distribution and AMPA receptor subunit composition in living hippocampal neurons. *J Neurosci* **20**, 7922-31. (2000).
68. Monyer, H., Seeburg, P.H. & Wisden, W. Glutamate-Operated Channels - Developmentally Early and Mature Forms Arise by Alternative Splicing. *Neuron* **6**, 799-810 (1991).
69. Allen, N.J. Role of glia in developmental synapse formation. *Curr Opin Neurobiol* **23**, 1027-33 (2013).
70. Suzuki, E., Kessler, M. & Arai, A.C. The fast kinetics of AMPA GluR3 receptors is selectively modulated by the TARPs gamma 4 and gamma 8. *Mol Cell Neurosci* **38**, 117-23 (2008).
71. Blair, M.G. et al. Developmental changes in structural and functional properties of hippocampal AMPARs parallels the emergence of deliberative spatial navigation in juvenile rats. *J Neurosci* **33**, 12218-28 (2013).
72. Mayer, M.L. Glutamate receptors at atomic resolution. *Nature* **440**, 456-62 (2006).
73. Sukumaran, M., Penn, A.C. & Greger, I.H. AMPA receptor assembly: atomic determinants and built-in modulators. *Adv Exp Med Biol* **970**, 241-64 (2012).
74. Nakagawa, T. The biochemistry, ultrastructure, and subunit assembly mechanism of AMPA receptors. *Mol Neurobiol* **42**, 161-84 (2010).
75. Rossmann, M. et al. Subunit-selective N-terminal domain associations organize the formation of AMPA receptor heteromers. *EMBO J* **30**, 959-71 (2011).
76. Greger, I.H., Khatri, L. & Ziff, E.B. RNA editing at arg607 controls AMPA receptor exit from the endoplasmic reticulum. *Neuron* **34**, 759-72 (2002).
77. Greger, I.H., Khatri, L., Kong, X. & Ziff, E.B. AMPA receptor tetramerization is mediated by Q/R editing. *Neuron* **40**, 763-74 (2003).
78. Mah, S.J., Cornell, E., Mitchell, N.A. & Fleck, M.W. Glutamate receptor trafficking: endoplasmic reticulum quality control involves ligand binding and receptor function. *J Neurosci* **25**, 2215-25 (2005).
79. Greger, I.H., Ziff, E.B. & Penn, A.C. Molecular determinants of AMPA receptor subunit assembly. *Trends Neurosci* **30**, 407-16 (2007).

80. Brorson, J.R., Li, D. & Suzuki, T. Selective expression of heteromeric AMPA receptors driven by flip-flop differences. *J Neurosci* **24**, 3461-70 (2004).
81. Greger, I.H., Akamine, P., Khatri, L. & Ziff, E.B. Developmentally regulated, combinatorial RNA processing modulates AMPA receptor biogenesis. *Neuron* **51**, 85-97 (2006).
82. Kumar, S.S., Bacci, A., Kharazia, V. & Huguenard, J.R. A developmental switch of AMPA receptor subunits in neocortical pyramidal neurons. *J Neurosci* **22**, 3005-15 (2002).
83. Lu, W., Khatri, L. & Ziff, E.B. Trafficking of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) receptor subunit GluA2 from the endoplasmic reticulum is stimulated by a complex containing Ca2+/calmodulin-activated kinase II (CaMKII) and PICK1 protein and by release of Ca2+ from internal stores. *J Biol Chem* **289**, 19218-30 (2014).
84. Tomita, S. et al. Functional studies and distribution define a family of transmembrane AMPA receptor regulatory proteins. *J Cell Biol* **161**, 805-16 (2003).
85. Vandenberghe, W., Nicoll, R.A. & Bredt, D.S. Interaction with the unfolded protein response reveals a role for stargazin in biosynthetic AMPA receptor transport. *J Neurosci* **25**, 1095-102 (2005).
86. Tomita, S., Nicoll, R.A. & Bredt, D.S. PDZ protein interactions regulating glutamate receptor function and plasticity. *J Cell Biol* **153**, F19-24. (2001).
87. Bokel, C., Dass, S., Wilsch-Brauninger, M. & Roth, S. Drosophila Cornichon acts as cargo receptor for ER export of the TGFalpha-like growth factor Gurken. *Development* **133**, 459-70 (2006).
88. Sauvageau, E. et al. CNIH4 interacts with newly synthesized GPCR and controls their export from the endoplasmic reticulum. *Traffic* **15**, 383-400 (2014).
89. Brockie, P.J. et al. Cornichons control ER export of AMPA receptors to regulate synaptic excitability. *Neuron* **80**, 129-42 (2013).
90. Harmel, N. et al. AMPA receptors commandeer an ancient cargo exporter for use as an auxiliary subunit for signaling. *PLoS One* **7**, e30681 (2012).
91. Hanley, J.G. Subunit-specific trafficking mechanisms regulating the synaptic expression of Ca^{2+}-permeable AMPA receptors. *Semin Cell Dev Biol* **27**, 14-22 (2014).
92. Terashima, A. et al. An essential role for PICK1 in NMDA receptor-dependent bidirectional synaptic plasticity. *Neuron* **57**, 872-82 (2008).
93. Lin, D.T. & Huganir, R.L. PICK1 and phosphorylation of the glutamate receptor 2 (GluR2) AMPA receptor subunit regulates GluR2 recycling after NMDA receptor-induced internalization. *J Neurosci* **27**, 13903-8 (2007).
94. Citri, A. et al. Calcium binding to PICK1 is essential for the intracellular retention of AMPA receptors underlying long-term depression. *J Neurosci* **30**, 16437-52 (2010).
95. Volk, L., Kim, C.H., Takamiya, K., Yu, Y. & Huganir, R.L. Developmental regulation of protein interacting with C kinase 1 (PICK1) function in hippocampal synaptic plasticity and learning. *Proc Natl Acad Sci U S A* **107**, 21784-9 (2010).
96. Petrini, E.M. et al. Endocytic trafficking and recycling maintain a pool of mobile surface AMPA receptors required for synaptic potentiation. *Neuron* **63**, 92-105 (2009).
97. Schnell, E. et al. Direct interactions between PSD-95 and stargazin control synaptic AMPA receptor number. *Proc Natl Acad Sci U S A* **99**, 13902-7 (2002).
98. Tomita, S. et al. Stargazin modulates AMPA receptor gating and trafficking by distinct domains. *Nature* **435**, 1052-8 (2005).

99. Boehm, J., Ehrlich, I., Hsieh, H. & Malinow, R. Two mutations preventing PDZ-protein interactions of GluR1 have opposite effects on synaptic plasticity. *Learn Mem* **13**, 562-5 (2006).
100. Mauceri, D., Cattabeni, F., Di Luca, M. & Gardoni, F. Calcium/calmodulin-dependent protein kinase II phosphorylation drives synapse-associated protein 97 into spines. *J Biol Chem* **279**, 23813-21 (2004).
101. Wu, H., Nash, J.E., Zamorano, P. & Garner, C.C. Interaction of SAP97 with minus-end-directed actin motor myosin VI. Implications for AMPA receptor trafficking. *J Biol Chem* **277**, 30928-34 (2002).
102. Loo, L.S., Tang, N., Al-Haddawi, M., Dawe, G.S. & Hong, W. A role for sorting nexin 27 in AMPA receptor trafficking. *Nat Commun* **5**, 3176 (2014).
103. Hussain, N.K., Diering, G.H., Sole, J., Anggono, V. & Huganir, R.L. Sorting Nexin 27 regulates basal and activity-dependent trafficking of AMPARs. *Proc Natl Acad Sci U S A* **111**, 11840-5 (2014).
104. Liu, S.J. & Cull-Candy, S.G. Subunit interaction with PICK and GRIP controls Ca2+ permeability of AMPARs at cerebellar synapses. *Nat Neurosci* **8**, 768-75 (2005).
105. Gardner, S.M. et al. Calcium-permeable AMPA receptor plasticity is mediated by subunit-specific interactions with PICK1 and NSF. *Neuron* **45**, 903-15 (2005).
106. Dong, H. et al. GRIP: a synaptic PDZ domain-containing protein that interacts with AMPA receptors. *Nature* **386**, 279-84 (1997).
107. Nishimune, A. et al. NSF binding to GluR2 regulates synaptic transmission. *Neuron* **21**, 87-97 (1998).
108. Jackson, A.C. & Nicoll, R.A. The expanding social network of ionotropic glutamate receptors: TARPs and other transmembrane auxiliary subunits. *Neuron* **70**, 178-99 (2011).
109. Haering, S.C., Tapken, D., Pahl, S. & Hollmann, M. Auxiliary subunits: shepherding AMPA receptors to the plasma membrane. *Membranes (Basel)* **4**, 469-90 (2014).
110. Zheng, Y. et al. SOL-1 is an auxiliary subunit that modulates the gating of GLR-1 glutamate receptors in Caenorhabditis elegans. *Proc Natl Acad Sci U S A* **103**, 1100-5 (2006).
111. Schwenk, J. et al. Functional proteomics identify cornichon proteins as auxiliary subunits of AMPA receptors. *Science* **323**, 1313-9 (2009).
112. Kalashnikova, E. et al. SynDIG1: an activity-regulated, AMPA- receptor-interacting transmembrane protein that regulates excitatory synapse development. *Neuron* **65**, 80-93 (2010).
113. Kirk, L.M. et al. Distribution of the SynDIG4/Proline rich transmembrane protein 1 in rat brain. *J Comp Neurol* (2015).
114. von Engelhardt, J. et al. CKAMP44: a brain-specific protein attenuating short-term synaptic plasticity in the dentate gyrus. *Science* **327**, 1518-22 (2010).
115. Farrow, P. et al. Auxiliary subunits of the CKAMP family differentially modulate AMPA receptor properties. *Elife* **4** (2015).
116. Shanks, N.F. et al. Differences in AMPA and kainate receptor interactomes facilitate identification of AMPA receptor auxiliary subunit GSG1L. *Cell Rep* **1**, 590-8 (2012).
117. Constals, A. et al. Glutamate-induced AMPA receptor desensitization increases their mobility and modulates short-term plasticity through unbinding from Stargazin. *Neuron* **85**, 787-803 (2015).
118. Morimoto-Tomita, M. et al. Autoinactivation of neuronal AMPA receptors via glutamate-regulated TARP interaction. *Neuron* **61**, 101-12 (2009).
119. Nakagawa, T., Cheng, Y., Ramm, E., Sheng, M. & Walz, T. Structure and different conformational states of native AMPA receptor complexes. *Nature* **433**, 545-9 (2005).

120. Semenov, A., Moykkynen, T., Coleman, S.K., Korpi, E.R. & Keinanen, K. Autoinactivation of the stargazin-AMPA receptor complex: subunit-dependency and independence from physical dissociation. *PLoS One* **7**, e49282 (2012).
121. Soto, D., Coombs, I.D., Kelly, L., Farrant, M. & Cull-Candy, S.G. Stargazin attenuates intracellular polyamine block of calcium-permeable AMPA receptors. *Nat Neurosci* **10**, 1260-7 (2007).
122. Soto, D. et al. Selective regulation of long-form calcium-permeable AMPA receptors by an atypical TARP, gamma-5. *Nat Neurosci* **12**, 277-85 (2009).
123. Bats, C., Soto, D., Studniarczyk, D., Farrant, M. & Cull-Candy, S.G. Channel properties reveal differential expression of TARPed and TARPless AMPARs in stargazer neurons. *Nat Neurosci* **15**, 853-61 (2012).
124. Jackson, A.C. et al. Probing TARP modulation of AMPA receptor conductance with polyamine toxins. *J Neurosci* **31**, 7511-20 (2011).
125. Studniarczyk, D., Coombs, I., Cull-Candy, S.G. & Farrant, M. TARP gamma-7 selectively enhances synaptic expression of calcium-permeable AMPARs. *Nat Neurosci* **16**, 1266-74 (2013).
126. Herring, B.E. et al. Cornichon proteins determine the subunit composition of synaptic AMPA receptors. *Neuron* **77**, 1083-96 (2013).
127. Chater, T.E. & Goda, Y. The role of AMPA receptors in postsynaptic mechanisms of synaptic plasticity. *Front Cell Neurosci* **8**, 401 (2014).
128. Wang, J.Q. et al. Roles of subunit phosphorylation in regulating glutamate receptor function. *Eur J Pharmacol* **728**, 183-7 (2014).
129. Lee, H.K., Barbarosie, M., Kameyama, K., Bear, M.F. & Huganir, R.L. Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature* **405**, 955-9 (2000).
130. Lu, W. & Roche, K.W. Posttranslational regulation of AMPA receptor trafficking and function. *Curr Opin Neurobiol* **22**, 470-9 (2012).
131. Roche, K.W., O'Brien, R.J., Mammen, A.L., Bernhardt, J. & Huganir, R.L. Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. *Neuron* **16**, 1179-88 (1996).
132. Kristensen, A.S. et al. Mechanism of Ca2+/calmodulin-dependent kinase II regulation of AMPA receptor gating. *Nat Neurosci* **14**, 727-35 (2011).
133. Banke, T.G. et al. Control of GluR1 AMPA receptor function by cAMP-dependent protein kinase. *J Neurosci* **20**, 89-102 (2000).
134. Lee, H.K., Takamiya, K., He, K., Song, L. & Huganir, R.L. Specific roles of AMPA receptor subunit GluR1 (GluA1) phosphorylation sites in regulating synaptic plasticity in the CA1 region of hippocampus. *J Neurophysiol* **103**, 479-89 (2010).
135. Steinberg, J.P. et al. Targeted in vivo mutations of the AMPA receptor subunit GluR2 and its interacting protein PICK1 eliminate cerebellar long-term depression. *Neuron* **49**, 845-60 (2006).
136. Luchkina, N.V. et al. Developmental switch in the kinase dependency of long-term potentiation depends on expression of GluA4 subunit-containing AMPA receptors. *Proc Natl Acad Sci U S A* **111**, 4321-6 (2014).
137. Henley, J.M., Craig, T.J. & Wilkinson, K.A. Neuronal SUMOylation: mechanisms, physiology, and roles in neuronal dysfunction. *Physiol Rev* **94**, 1249-85 (2014).
138. Sanderson, J.L. et al. AKAP150-anchored calcineurin regulates synaptic plasticity by limiting synaptic incorporation of Ca2+-permeable AMPA receptors. *J Neurosci* **32**, 15036-52 (2012).
139. Kim, S. & Ziff, E.B. Calcineurin mediates synaptic scaling via synaptic trafficking of Ca2+-permeable AMPA receptors. *PLoS Biol* **12**, e1001900 (2014).

140. Diering, G.H., Gustina, A.S. & Huganir, R.L. PKA-GluA1 coupling via AKAP5 controls AMPA receptor phosphorylation and cell-surface targeting during bidirectional homeostatic plasticity. *Neuron* **84**, 790-805 (2014).
141. Guire, E.S., Oh, M.C., Soderling, T.R. & Derkach, V.A. Recruitment of calcium-permeable AMPA receptors during synaptic potentiation is regulated by CaM-kinase I. *J Neurosci* **28**, 6000-9 (2008).
142. Hampel, H. et al. The future of Alzheimer's disease: the next 10 years. *Prog Neurobiol* **95**, 718-28 (2011).
143. Shankar, G.M. et al. Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med* **14**, 837-42 (2008).
144. Walsh, D.M. & Selkoe, D.J. A beta oligomers - a decade of discovery. *J Neurochem* **101**, 1172-84 (2007).
145. Zhao, W.Q. et al. Inhibition of calcineurin-mediated endocytosis and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors prevents amyloid beta oligomer-induced synaptic disruption. *J Biol Chem* **285**, 7619-32 (2010).
146. Whitcomb, D.J. et al. Intracellular oligomeric amyloid-beta rapidly regulates GluA1 subunit of AMPA receptor in the hippocampus. *Sci Rep* **5**, 10934 (2015).
147. Spaethling, J.M., Klein, D.M., Singh, P. & Meaney, D.F. Calcium-permeable AMPA receptors appear in cortical neurons after traumatic mechanical injury and contribute to neuronal fate. *J Neurotrauma* **25**, 1207-16 (2008).
148. Grooms, S.Y., Opitz, T., Bennett, M.V. & Zukin, R.S. Status epilepticus decreases glutamate receptor 2 mRNA and protein expression in hippocampal pyramidal cells before neuronal death. *Proc Natl Acad Sci U S A* **97**, 3631-6 (2000).
149. Yamashita, T. & Kwak, S. The molecular link between inefficient GluA2 Q/R site-RNA editing and TDP-43 pathology in motor neurons of sporadic amyotrophic lateral sclerosis patients. *Brain Res* **1584**, 28-38 (2014).
150. Sommer, B., Kohler, M., Sprengel, R. & Seeburg, P.H. RNA Editing in Brain Controls a Determinant of Ion Flow in Glutamate-Gated Channels. *Cell* **67**, 11-19 (1991).
151. Verdoorn, T.A., Burnashev, N., Monyer, H., Seeburg, P.H. & Sakmann, B. Structural Determinants of Ion Flow Through Recombinant Glutamate Receptor Channels. *Science* **252**, 1715-1718 (1991).
152. Hestrin, S. Different Glutamate Receptor Channels Mediate Fast Excitatory Synaptic Currents in Inhibitory and Excitatory Cortical Neurons. *Neuron* **11**, 1083-1091 (1993).
153. Clem, R.L. & Barth, A. Pathway-specific trafficking of native AMPARs by in vivo experience. *Neuron* **49**, 663-70 (2006).
154. Goel, A. et al. Cross-modal regulation of synaptic AMPA receptors in primary sensory cortices by visual experience. *Nat Neurosci* **9**, 1001-3 (2006).
155. Goel, A. et al. Phosphorylation of AMPA receptors is required for sensory deprivation-induced homeostatic synaptic plasticity. *PLoS One* **6**, e18264 (2011).
156. Clem, R.L., Anggono, V. & Huganir, R.L. PICK1 regulates incorporation of calcium-permeable AMPA receptors during cortical synaptic strengthening. J Neurosci 30, 6360-6 (2010).
157. Bellone, C. & Luscher, C. mGluRs induce a long-term depression in the ventral tegmental area that involves a switch of the subunit composition of AMPA receptors. Eur J Neurosci 21, 1280-8 (2005).
158. Bellone, C. & Luscher, C. Cocaine triggered AMPA receptor redistribution is reversed in vivo by mGluR-dependent long-term depression. Nat Neurosci 9, 636-41 (2006).

159. Conrad, K.L. et al. Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. Nature 454, 118-21 (2008).

Boxes

Box 1: **Synaptic AMPAR composition – the importance of the GluA2 subunit:**

AMPAR subunit composition is a crucial determinant of the conductance, trafficking and calcium-permeability of the receptors. Primarily, these properties are conferred by the presence or absence of the GluA2 subunit. In the brain, the vast majority (>99%) of GluA2 mRNA exists in an edited form, resulting in a change from glutamine to arginine at position 607 [150]. This alteration of charge, which occurs in the channel pore, blocks the passage of Ca^{2+} ions, prevents the channel from being blocked by intracellular polyamines, and reduces the single-channel conductance of the receptor [151]. Notably, as discussed in the main text, Q/R editing at position 607 also alters the trafficking properties of GluA2-containing receptors through the biosynthetic pathway. Thus, AMPARs lacking GluA2, or containing unedited GluA2, are calcium permeable, show higher single-channel conductance, and are inwardly-rectifying due to voltage-dependent block by intracellular polyamines, while receptors containing GluA2 are calcium impermeable, and exhibit a lower single-channel conductance and linear voltage-current relationship [152].

Box 2 **Functional roles of CP-AMPAR trafficking in plasticity *in vivo***

Consistent with an important role for selective CP-AMPAR trafficking in brain function, a growing number of studies have observed alterations in CP-AMPAR trafficking *in vivo*.

- Single whisker stimulation in the absence of adjacent whiskers enhances spared whisker responses in the neocortex, and leads to a potentiation of AMPAR EPSCs, through incorporation of GluA2-lacking CP-AMPARs [153].
- Visual depravation through dark-rearing rats increases AMPAR EPSCs at layer 2/3 pyramidal neurons in visual cortex but reduces them in somatosensory and auditory cortices [154]. The *in vivo* homeostatic upscaling in the visual cortex is mediated via increased CP-AMPARs and is absent in GluA1-S845A mice [155].
- In fear conditioning a conditioned stimulus (e.g. sound) is paired with an adverse stimuli (e.g. foot shock). Following training, a fear response is elicited to the conditioned stimulus alone. This requires GluA1-S845 phosphorylation and recruitment of CP-AMPARs at thalamic synapses on amygdala neurons [156]. Fear extinction occurs through mGluR1-mediated removal of CP-AMPARs at these synapses [156].
- The mechanisms of addiction also involve CP-AMPAR trafficking. At glutamatergic synapses onto dopaminergic neurons in the ventral tegmental area a single cocaine administration drives PICK1-dependent synaptic insertion of GluA2-lacking CP-AMPARs [157, 158]. As with fear extinction, this is reversed by mGluR1 activation, suggesting mGluR1-dependent LTD may represent a general mechanism to reverse CP-AMPAR upregulation [157]. Similarly, CP-AMPARs increase in the nucleus accumbens (NAc) in rats during cocaine withdrawal and their blockade reduces cue-induced cocaine seeking, consistent with roles in addiction [159].

Thus, there is strong evidence that CP-AMPARs play a critical role in *in vivo*. Overall, synaptic CP-AMPARs can tune neuronal excitability to induce plasticity and their activity-dependent removal can define a time-frame in which memories can be erased. These changes parallel observations in *in vitro* systems, whereby plasticity

induced by CP-AMPAR incorporation remains transient until they are ultimately replaced by CI-AMPARs.

Figure Legends

Figure 1: AMPAR receptor topology and properties. A) Schematic depicting the membrane topology of an AMPA receptor subunit. Each subunit has an extracellular N-terminal glutamate binding site, three full transmembrane domains, an intracellular re-entrant loop, which is the site of Q607R editing in the GluA2 subunit, and an intracellular C-terminus. The subunits combine to form tetramers, and most AMPARs are heterotetrameric. B) AMPARs lacking GluA2 subunits (non-GluA2 subunits are denoted as cream) are calcium-permeable. Similarly, AMPARs containing the GluA2 subunit (light blue) in its unedited (Q, in red) form also gate calcium. However, the presence of a GluA2 subunit RNA edited to replace Q607 with an arginine (R, dark blue) renders the AMPAR impermeable to calcium. C) Sequences of the intracellular C-terminal tails of the predominant isoforms of human GluA1 and GluA2, indicating interaction and posttranslational modification sites. Key serine residues that can be phosphorylated by CaMKII and PKC are shown in pink. PDZ ligand sequences are shaded in green and important PDZ protein interactors for GluA1 and GluA2 listed. The residues in the GluA2 sequence shaded grey represent where NSF and AP2 proteins interact to regulate endocytosis.

Figure 2: The slot hypothesis of LTP. A widely-accepted model of LTP suggests that AMPARs laterally diffuse within the plasma membrane and are trapped at synapses by slot proteins at the postsynaptic membrane. Before LTP induction (left), AMPARs are localised to PSD95-containing nanodomains that are anchored to the actin cytoskeleton in spines. The AMPARs are retained within these domains through interactions between TARPs and PSD95 but they are continually repositioned within the PSD because of constitutive actin filament dynamics. Extrasynaptic AMPARs laterally diffuse within the membrane and the number of surface expressed AMPARs is maintained by constitutive recycling in the spine and dendrite.
Induction of LTP increases the AMPAR binding capacity of the slot proteins, potentially through phosphorylation (denoted by the red P) of the TARP Stargazin enhancing its binding to PSD95. This results in entrapment of laterally diffusing AMPARs and potentiation of the synapse. In addition, net AMPAR exocytosis (denoted by bold arrow) replenishes the pool of extrasynaptic receptors.

Figure 3: Subunit-specific AMPAR trafficking through the secretory pathway. AMPARs are synthesized and assembled in the ER, which extends throughout the neuron. The stoichiometry of assembly and ER exit are highly regulated. GluA1-containing AMPARs or AMPARs containing unedited GluA2 traffic readily through the ER to the cell surface. However, edited GluA2 is ER retained, resulting in a stable ER-resident pool of unassembled GluA2, but this retention can be overcome when they assemble into heteromeric receptors containing both GluA1 and GluA2. ER release of GluA2-containing AMPARs can be controlled by CamKII and PICK1 in an activity-dependent manner via increased intracellular calcium, and is also strongly influenced by association with auxiliary proteins (not shown). From the ER, assembled AMPARs progress to the Golgi and then on to the plasma membrane, where they undergo lateral diffusion and can be retained at synapses. In addition to this route through the secretory pathway, local translation of AMPARs utilising ER in dendrites and, potentially, dendritic Golgi outposts (not shown), can provide a rapid source of AMPARs under activity-dependent conditions.

Figure 4: Ca^{2+}-permeable AMPAR (CP-AMPAR) trafficking in hippocampal plasticity. Under basal conditions synaptic AMPARs are predominantly calcium-impermeable, and are anchored to PSD95 and the actin cytoskeleton. They undergo both lateral diffusion in the membrane and rapid constitutive recycling. Furthermore, under some conditions, CP-AMPARs can be observed extrasynaptically. During LTP induction, activation of PKA, which is anchored at synapses by the scaffold protein AKAP150, leads to phosphorylation (indicated by a red 'P') of GluA1 at Ser845 in both spines and dendrites, promoting its surface expression and leading to the appearance of synaptic CP-AMPARs. In addition, enhanced binding of PICK1 to GluA2-containing CI-AMPARs in intracellular vesicles restricts their recycling to the cell surface, thus further enhancing the ratio of CP to CI-AMPARs. During activity depravation to induce synaptic homeostatic scaling, calcineurin (CaN) activity is reduced, favouring PKA-mediated phosphorylation of GluA1 subunits at Ser845, and promoting surface expression of GluA1-containing AMPARs and their synaptic incorporation. Furthermore, induction of homeostatic scaling via NMDAR-blockade promotes local translation of GluA1 in dendrites, and an increase in surface expression of GluA1-containing CP-AMPARs.
By contrast, LTD is associated with dephosphorylation of GluA1 at Ser845, reducing the extrasynaptic pool of GluA1-containing CP-AMPARs, which then undergo lysosomal degradation.

Figure 5: CP-AMPAR plasticity at cerebellar granule cell-stellate cell synapses. Unusually, in cerebellar stellate cells, postsynaptic AMPARs are primarily calcium-permeable GluA3 homomers, which are anchored at synapses through interactions with GRIP1. In contrast, extrasynaptic AMPARs are calcium-impermeable. However, repetitive synaptic activity induces a switch in synaptic AMPARs from calcium permeable to calcium impermeable through uncoupling of the CP-AMPARs from GRIP1, resulting in diffusion of the CP-AMPARs from the synapse, and their replacement by extrasynaptic CI-AMPARs. PICK1 and NSF are also thought to play a role in this switch, through supplying extrasynaptic CI-AMPARs and stabilising surface expressed CI-AMPARs, respectively.

Biographies

Jeremy Henley

Jeremy Henley obtained his PhD from King's College London and did postdoctoral training at Cornell University and the MRC-LMB in Cambridge. He was lecturer at the Birmingham University, did a sabbatical at Kyoto University and is now Professor of Molecular Neuroscience at Bristol University. His research interests encompass the molecular, protein and cell biology of synaptic function. He has significantly advanced understanding of the trafficking and regulation of AMPAR and kainate receptors. His work on neuronal SUMOylation has shown that SUMOylation is a fundamental regulator of synaptic function and dysfunction in health and neurodegenerative disease.

Kevin Wilkinson

Kevin Wilkinson obtained his PhD from Bristol University in 2010 and is currently an MRC-funded research associate. His core research focus concerns how multiple interactors and post-translational modifications interact to orchestrate receptor trafficking. His research highlights include the discovery and characterization of multiple novel neuronal substrates for post-translational protein modification by SUMO. His wider ambition is to understand how synaptic protein trafficking operates

under normal conditions, and how these pathways are perturbed in neurological disorders.

Glossary

AKAP150 - (aka AKAP79 in humans)
A specialized scaffold protein that can bring together protein kinase A (PKA), protein kinase C (PKC), the scaffolding proteins SAP97 and PSD95 and the Ca^{2+}-dependent protein phosphatase calcineurin/PP2B with AMPARs at synapses.

Auxiliary subunits
Specialized, transmembrane components of the AMPAR complex that modulate forward trafficking and the pharmacological and functional properties of the surface expressed receptor.

CI-AMPAR
Ca^{2+}-impermeable AMPAR which is a tetrameric assembly containing the RNA-edited form of the GluA2 subunit in which the uncharged amino acid glutamine (Q) to the positively charged arginine (R) in the ion channel.

Calcium permeable-AMPAR
AMPAR are calcium permeable when they lack GluA2 or containing unedited GluA2.

Homeostatic scaling
A feedback mechanism whereby a neuron can up- or down-regulate its synaptic responsiveness in response to sustained alterations in activity.

Ionotropic glutamate receptor
A family of glutamate-gated cation channels which, based on pharmacological properties, can be subdivided into AMPA, NMDA and kainate receptors.

LTD
Long-term depression, which produces a long-lasting decrease in synaptic strength mainly due to reduced numbers of postsynaptic AMPARs.

LTP
Long-term potentiation is the persistent strengthening of synaptic transmission due mainly to increased numbers of postsynaptic AMPARs

MAGUK
Membrane-associated guanylate kinases are a superfamily of multidomain, catalytically inert scaffolding proteins that facilitate interactions between cytoskeletal proteins, microtubule/actin based machinery and molecules involved in signal transduction.

PDZ domain - a structural domain of 80-90 amino-acids that binds cognate proteins containing a short C-terminal PDZ ligand. Among other functions, PDZ interactions anchor receptor proteins in the membrane to cytoskeletal components.

RNA editing
A post-transcriptional modification that changes an RNA molecule to insert, delete or substitute nucleotides. Editing of the base A→I at a specific site result in the substitution Q with R in almost all GluA2 subunits in CNS.

Silent synapse –
A synapse that contains postsynaptic NMDARs but lacks AMPARs, rendering the synapse silent at resting membrane potential.

Synaptic plasticity
The process by which synaptic transmission can strengthen or weaken in response to specific patterns of activity

Online summary

- AMPARs mediate nearly all fast excitatory neurotransmission in the mammalian CNS.

- AMPARs are heteromeric assemblies of four core subunits, GluA1-4 together with auxiliary subunits and a dynamically changing set of interacting proteins.

- The assembly and subunit composition of AMPARs is activity-dependently regulated during biogenesis.

- The presence or absence of the edited from of the GluA2 subunit, GluA2(R), determines whether the assembled AMPAR receptor gates Ca^{2+}.

- There is a wealth of evidence that the synaptic trafficking, retention and removal of AMPARs of specific subunit combinations and with specific biophysical properties is of paramount importance for synaptic plasticity. These AMPAR-subtype specific events are regulated by both protein interactions and phosphorylation events within the C-terminal tails.

- Recent studies reporting that the C-terminal tails are not essential for plasticity and that very few GluA1 subunits are phosphorylated have prompted a major re-evaluation of the fundamental mechanisms of AMPAR trafficking and synaptic plasticity.

- Understanding the molecular details of AMPAR assembly, trafficking, recycling and degradation, and how dysfunction impacts on synapses, neurons and networks will provide invaluable insight into neurological and neurodegerative disease.